REVIEW ARTICLE

The epigenetics of normal pregnancy

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Summary: Epigenetic modifications to chromatin are essential for the specification and maintenance of cell fate, enabling the same genome to programme a variety of cellular outcomes. Epigenetic modulation of gene expression is also a critical mechanism by which cells stabilize their responses to environmental stimuli, including both nutritional cues and hormonal signalling. Unsurprisingly, epigenetics is proving to be vitally important in fetal development, and this review addresses our current understanding of the roles of epigenetic regulation in the prenatal phase. It is striking that while there has been a major interest in the intersection of fetal health with epigenetics, there has been relatively little discussion in the literature on epigenetic changes in the pregnant woman, and we attempt to redress this balance, drawing on the fragmented but intriguing experimental literature in this field.

Keywords: endocrinology, metabolism, nutrition, physiology

INTRODUCTION TO EPIGENETICS

Epigenetic traits have been defined operationally as 'stably heritable phenotypes resulting from changes in a chromosome without alterations in the DNA sequence'.[1] Epigenetic modifications (also known as epigenetic marks) form a network of covalent alterations to DNA and histone proteins (Figure 1) which, in turn, interacts with other cellular proteins, typically in multicomponent mediator complexes. The end result is regulation of gene expression. There are a number of excellent recent reviews of the molecular biology of epigenetic control of gene expression.[2,3]

Developmental biology is arguably the discipline that has had the greatest impact on spotlighting the fundamental nature of epigenetic control of cell fate. From the amphibian somatic cell nuclear transfer experiments of John Gurdon starting in the 1960s, through Azim Surani's ground-breaking studies on the non-equivalence of the maternal and paternal genomes, right up to the current excitement following Shinya Yamanaka's creation of induced pluripotent stem cells, developmental biology has been at the forefront of the great conceptual breakthroughs in epigenetics.

The regulation of gene expression via epigenetic modifications may be relatively short-term and dynamic, or may be exceptionally stable if the chromatin modifications lead to the hypermethylated DNA state associated with the formation of transcriptionally silent heterochromatin. It is clear that epigenetics plays a major role in embryonic development. The transient modifications function in response to signalling molecules to convert relatively undifferentiated cells into increasingly specialized moieties. The gene expression patterns can be stabilized by the development of differential DNA methylation of specific genes, locking cells into a particular fate. These processes are absolutely essential in the generation of a new multitrillion celled human from a single-celled pluripotent zygote.[4]

Pregnancy is guided by a well-known interaction of hormonal changes which not only ensures that key features of the developmental process (such as placentation) occur at the right time but also that parturition occurs once the baby's development is complete. Acting in concert with the underlying hormonal aspects of pregnancy is the complex interplay of epigenetic modifications, whose appropriate integration and critical timing are also vital for a successful pregnancy.

Molecular epigenetics has struggled to elucidate the key mediators of all these stages. When we consider the size and complexity of the human genome and epigenome, in which there are multiple possible combinations of epigenetic modifications at any given chromatin locus, the reasons for this become obvious. However, recent technological advances are generating enormous amounts of data that are opening windows into the control of fetal development. There has been much less attention paid to the effects of pregnancy on the mother, even though epigenetic modifications to chromatin are clearly an important component of long-term responses to hormonal signalling.

In this review, we attempt to provide an overview of the recent advances in our understanding of the molecular epigenetic regulation of pregnancy, with respect to both the fetal and maternal perspective. We do not address the topic of imprinting, as a number of excellent reviews already exist for this aspect of developmental epigenetics.[5,6] Our focus in this review is on healthy pregnancy. The role of epigenetic processes in abnormal pregnancy will be the subject of a separate review.

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EPIGENETIC INFLUENCES ON FETAL DEVELOPMENT

Ovulation

Clearly, ovulation does not always result in pregnancy, but the former is a prerequisite for the latter, so it is instructive to

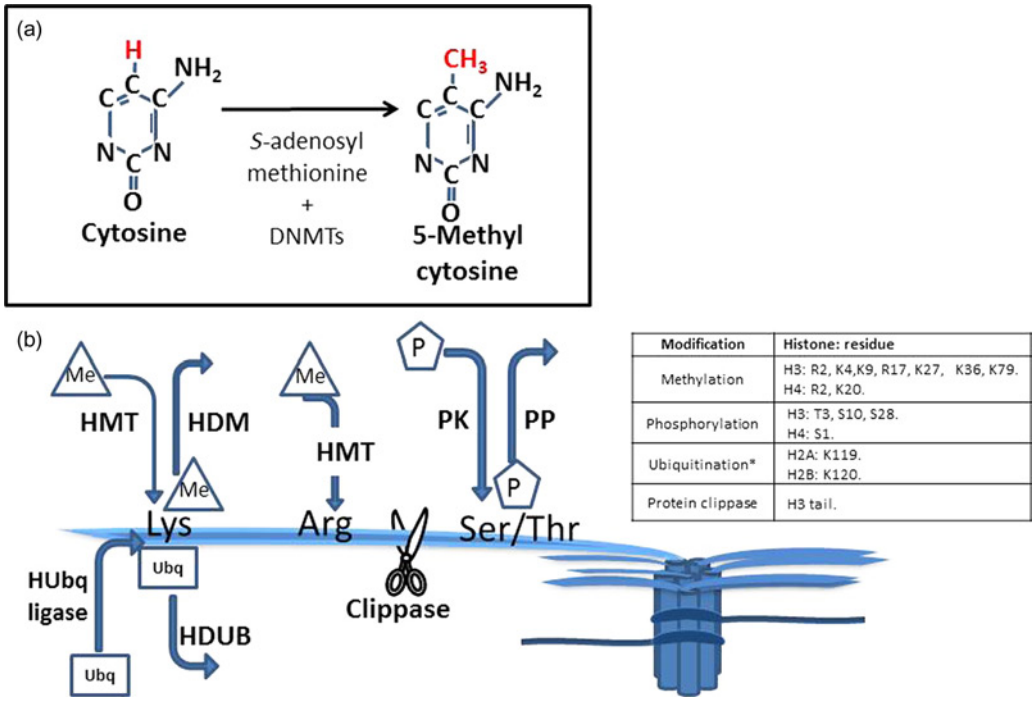

Figure 1 **Mechanisms of action of different epigenetic enzymes. (a) DNA methylation of cytosine residue. (b) Histone-modifying enzymes. HDM, histone demethylases; HMT, histone methyltransferases; HUbq ligase, histone ubiquitin ligases; HDUB, histone deubiquitinases; PK, protein kinase; PP, protein phosphotase; Me, methyl groups, P, phosphate groups. Inset table shows the residues known to be modified on their respective histones. *Ubiquitination refers to the signalling processes involved in transcription initiation and elongation, silencing, DNA repair rather than the degradation**

consider the epigenetics of this precursor to the pregnant state. The first stage of pregnancy requires the production of a viable oocyte from the ovaries, its fusion with a sperm cell and implantation into the wall of the uterus. Oocyte production begins in the primordial germ cells and requires erasure of the majority of established somatic epigenomic modifications.[3] In mice, the histone methyltransferase (HMT) MLL2 is required for female fertility and development of oocytes particularly postnatally. MLL2 generates trimethylation of lysine position 4 on histone H3 (H3K4me3), a modification that is associated with activation of gene expression. The MLL2 protein is expressed at high levels in the developing oocytes.[7] Its loss via a conditional knockout results in female mice which fail to ovulate. Maternally contributed expression of epigenetic enzymes is not restricted to MLL2. For example, the protein arginine methyltransferase, PRMT5, which generates repressive histone modifications, is also supplied by the female gamete.[8] It is likely that such enzymes play key roles at very early points in development. This preloading with epigenetic enzymes is important because, once formed, the oocytes are transcriptionally silenced until fertilization.

As ovulation approaches, a number of epigenetic changes take place during the growth of oocytes with increases detected in DNA methylation along with a number of histone lysine acetylation and methylation events on histones H3 and H4.[9] These changes correlate with the presence of a number of histone acetyltransferase enzymes includingSrc-1, p300 and P-caf. The changes in histone methylation suggest possible roles for HMTs, Smyd3, Set7 and Mll2 proteins. However, there is not always a simple relationship between the level of expression of HMTs and the pattern of methylation at their histone targets. This has been demonstrated for the methylation of H3K9. G9a and Glp, which dimethylate H3K9, are present at high levels along with elevated levels of H3K9me2. However, Eset and Suv39h, which are responsible for trimethylation, are also present at high levels but levels of H3K9me3 are low. This suggests that specific roles of these enzymes are not purely driven by their levels of expression.

Preimplantation

After release of the mature oocyte and fertilization by the sperm cell, the newly formed zygote undergoes a number of developmental changes which results in the epigenetic reprogramming of the non-imprinted genes in the genome. This re-sets the epigenome to an expression status compatible with totipotency (the ability of a cell to form all cell types in the body) before implantation (reviewed in reference[10]). One of the key mechanisms, but which is least understood, is the initial large-scale erasure of DNA methylation marks from the paternal genome. This would appear to be an active process as it occurs before replication and cell division takes place. However the mechanism responsible for this (whether direct DNA demethylation or indirect changes via base excision response) remains controversial (fully detailed in reference[3]). Recent work in embryonic stem (ES) cells demonstrates this is most likely to involve hydroxylation of the methylated cytosine residue, mediated by the Tet family of enzymes.[11]

Following this epigenomic erasure, the epigenome is re-patterned with *de novo* establishment of histone and DNA modifications as cell lineages develop. This occurs from the

very earliest stages of cellular commitment, during formation of the blastocyst. This is functionally subdivided into the outer layer of differentiated cells (the trophoectoderm [TE]), surrounding the small cluster of cells in the inner cell mass (ICM) which contains the pluripotent cells. The ICM is the experimental source of ES cells. Some of the epigenetic mechanisms underlying this process are beginning to be revealed (reviewed in detail in reference[12]).

Much of the work studying this reprogramming has been conducted in rodents where the differentiation of these early cells into the TE and the ICM is correlated with the expression of Cdx2 and Oct4, respectively. The downstream effects of Oct4 activity are critically dependent on the establishment of repressive histone modifications by theH3K9 HMT Eset (also known as Setdb1). Eset is recruited by and co-associates with Oct4 to repress *Cdx2* via methylation of H3K9. Eset depletion causes embryonic lethality at the peri-implantation stage. This is associated with incomplete formation of the ICM, with the cells becoming more TE-like due to the de-repression of *Cdx2*.[13–15]

Extreme early embryonic lethality in response to disruption of key epigenetic mediators is not restricted to Eset. Knockout of *Prmt5* also results in exceptionally early loss of embryos.[8] For each of these genes, disruption of the development of the ICM is so severe that it is impossible to generate ES cells from the homozygous knockouts.

Timing of the action of these and other epigenetic enzymes is critical to normal embryo development. However, the precise roles of these various epigenetic agents in initiating, driving or maintaining developmental pathways remain the subject of intense scrutiny.[16]

Implantation and placentation

The next stage for the developing zygote is its implantation into the endometrial wall and the formation of the placenta. As described above, formation of the trophoblast cells is critically dependent on the up-regulation of Cdx2 (reviewed in reference[17]). One of the factors that influence Cdx2 expression is Elf5. In trophoblast cells, the promoter region of the Elf5 gene is hypomethylated, resulting in increased levels of Elf5 which drive up expression of Cdx2. This results in a positive feedback loop between Cdx2 and Elf5 expression where Elf5 drives Cdx2, and where Cdx2 in turn drives Elf5, resulting in trophoblast differentiation. In contrast, in the cells of the ICM, the DNA of the Elf5 promoter is hypermethylated, which shuts down Elf5 transcription, thereby preventing trophoblast formation.[18]

Unlike the cells that arise from the ICM, the genome of the placental precursors remains relatively free from *de novo* DNA methylation (reviewed in reference[19]). The key factors for causing DNA methylation of those genes in the placenta which do become methylated are still being elucidated,[20–22] but interestingly distinct changes are seen in histone methylation marks in the different cell lineages of the developing zygote.[23] Preimplantation, the repressive H3K27me3 mark, is present at relatively low levels in the TE and extra-ES stem cells compared with the ICM-derived ES cells. This is consistent with the relatively low levels of the enzymes that constitute the relevant methyltransferase machinery, the polycomb repressive complex 2. Postimplantation, the levels of H3K27me3 are relatively high in extraembryonic tissues. Together with the presence of other repressive marks in these tissues, such as H3K9me3, this suggests that these modifications are associated with the formation of specific cell lineages.[23]

Nutrition as an epigenetic stimulus in fetal development

It is during the first trimester that the majority of the fetal development occurs and this coincides with global changes to epigenetic modifications in the embryo.[3] How these widespread changes relate to specific pathways of fetal tissue development remains a key question. One productive area of research has been the differentiation of mesenchymal cells in chondrogenesis and osteogenesis.[24,25] These studies have revealed the importance of histone acetylation and DNA methylation modifications in mediating cellular differentiation.

Another area of great interest in relation to pregnancy is the impact of the environment on epigenetic control. For example, the formation and closure of the neural tube is a very important event in the embryo's development, and incomplete development can lead to malformations referred to as neural tube defects.[26] Deficiencies in the intake of folic acid have long been linked to neural tube defects. Intriguingly, recent studies have suggested this may have an epigenetic component (reviewed in reference[27]). Folic acid is required for the production of *S*-adenosyl methionine (SAM). SAM is the molecule that donates the methyl group when DNA methyltransferases modify DNA (see Figure 1a). Mice weaned on a diet low in folic acid develop abnormal regulation of imprinted regions of the genome,[28] demonstrating that the levels of this key nutrient do influence epigenetic signalling.

The effects of the nutritional status of the mother on fetal outcomes have been intensively studied, particularly since the landmark Dutch famine study.[29] This revealed significant effects on resulting adult obesity in the offspring (reviewed in reference[30]). These effects have been linked to DNA methylation events on a number of growth-related genes and recently to placental expression of micro-RNAs.[31] Similar to folic acid, decreases in maternal dietary choline intake negatively influence the availability of SAM as a DNA methyl donor, resulting in epigenetic changes to the offspring.[32]

EPIGENETIC CHANGES TO THE PREGNANT MOTHER

Oestrogen signalling

As the ovarian follicles mature, they secrete increasing amounts of oestrogens, and it is well documented that these hormones have a number of effects on the pregnant female.[33] Oestradiol, the predominant oestrogen, acts to enhance the effects of follicle stimulating hormone. This promotes follicle maturation and development of breast tissue, and initiates the proliferation of cells in the formation of the endometrium.[34–36] Much of the information regarding epigenetic interaction with oestrogen signalling has been derived from cancer cells lines.[37–40] However, the fact that both processes are strongly associated with the proliferation of cell numbers, albeit with entirely different endpoints, may allow us to draw parallels between these systems and give us some additional insights into the epigenetic regulation in response to the types of hormonal changes seen during pregnancy.

The effects of oestrogens are mediated by the alpha and beta oestrogen receptors. Following ligand binding, these translocate from the cell membrane to the nucleus, where they bind directly or as part of a multimeric complex to DNA. This generally promotes gene expression, but the effects can be further modulated by interaction with a number of epigenetic enzymes.[41,42] This results in the establishment of multimeric protein complexes, targeted to specific genomic locations, which leads to an epigenetic signalling cascade.

The interaction of epigenetic processes with hormonal signalling can be more complicated than a simple analysis of gene function might indicate. For example, KMT8 (as known as PRDM2 or RIZ1) methylates H3K9, generating a repressive chromatin mark. Unexpectedly, KMT8 acts as a co-activator with the oestrogen receptor. KMT8 knockout in female mice results in a number of phenotypes consistent with a decreased response to female sex hormones. In pregnant females these include a reduced increase in uterine weight, reduced epithelial vaginal thickening in response to oestrogen and reduced litter sizes compared with their wild-type counterparts.[43]

More recently, modifications generated by other HMTs have been shown to be important in the oestrogen signalling pathways. The arginine methyltransferases PRMT1, PRMT4 and PRMT6 which methylate histone proteins are each co-activators of receptor-mediated oestrogen signalling in breast-derived cell lines.[37,40] Among the lysine methyltransferases, SMYD3 and SET7 play co-activator roles with the oestrogen receptor alpha, when exposed to oestrogen. SMYD3 is responsible for H3K4 methylation, generating an activating chromatin modification and thereby facilitating oestrogen-mediated gene activation at ER-induced target genes.[38] SET7 may influence oestrogen signalling via a different mechanism. It has been reported that SET7 directly methylates the oestrogen receptor itself at lysine 302. This stabilizes the receptor, promoting its efficient recruitment to target genes.[39] This highlights an important additional layer of complexity, which must be considered when analysing the effects and roles of epigenetic enzymes. Many of these proteins have both histone and non-histone targets, and unravelling which of these modes of action is most critical in response to a specific stimulus can be challenging.

Successful implantation is critical for the continuing effective development of the embryo and adequate uterine receptivity plays a major role. The formation of the endometrium is important to this successful outcome and is linked to changes in the acetylation of histone lysine residues which occur during the menstrual cycle. Interestingly, the histone deacetylase inhibitor sodium valproate decreases the proliferation of an endometrial cell line, potentially linking this histone modification to endometrial formation (all above reviewed in reference[34]). The decidualization of the endometrial stromal cells which regulates the invasion of the trophoblast cells has been reported to be in part regulated by DNA methylation,[44,45] although any further role of DNA methylation in endometrial function has yet to be fully characterized.[46]

As pregnancy progresses, a number of overt changes become increasingly obvious in the mother. The breast tissue changes in preparation for lactation, a process that is largely underpinned by lactogenic hormones secreted via the anterior pituitary. However, there is evidence that many of these changes are mediated at least in part by epigenetic mechanisms. The promoters of the milk-related genes have low levels of DNA methylation in lactating mammary glands, allowing gene expression to take place. In non-mammary or non-lactating mammary tissue, these genes are hypermethylated at the DNA level (for a review see reference[47]). In the mammary glands of mice, levels of Dnmt1 and Dnmt3b3b are lower in lactating females compared with virgin and involuted females.[48]

Lactation is also linked to histone acetylation. The casein gene cluster shows high levels of histone acetylation in the mammary tissue of lactating mice. Such histone acetylation is typically associated with transcriptionally active genes.[47] Other studies have shown changes in nuclear architecture during lactation. H4K20me3 foci become more compact in lactating mammary epithelial cells and located more frequently at the nuclear periphery. In contrast, H4K20me3 foci are found throughout the nucleus in mammary epithelial cells from virgin and pregnant mice.[49]

CONCLUSIONS

It is clear that epigenetic regulation is a key feature of pregnancy and development, but our current understanding is rather piecemeal. In addition to the histone and DNA modifications that are described in this review, there are further aspects which are currently underexplored. These include the roles of non-canonical histone variants and the impact of regulatory non-coding RNAs, to name but two. In addition, most of the fundamental insights into the roles of epigenetics have been obtained from rodent species. While it is ethically and scientifically challenging to research reproduction in humans, it is essential that we gain a better mechanistic understanding of how these processes operate in human reproduction. This will be key for the improvement of both infant and maternal health, particularly in a more rational analysis of dietary effects in pregnancy. Furthermore, the only currently marketed drugs targeting epigenetic proteins are licensed in oncology. This is likely to change as epigenetic drug discovery progresses in chronic conditions. If such drugs are to be administered during pregnancy or to women of childbearing age, they will create a greater need for enhanced mechanistic understanding of epigenetic regulation of development and pregnancy.

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